

PRECLINICAL RESEARCH

Preservation of Ischemic Myocardial Function and Integrity With Targeted Cytoskeleton-Specific Immunoliposomes

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| OBJECTIVES | We sought to demonstrate preservation of myocardial function and integrity after targeted cytoskeleton-specific immunoliposome (CSIL) treatment of globally ischemic Langendorff instrumented hearts and a time response to treatment. |
| BACKGROUND | Cell membrane lesion sealing of hypoxic cardiocytes in culture with CSIL has been reported. |
| METHODS | Langendorff-perfused isolated rat hearts were subjected to global ischemia (25 min). Either CSIL or placebo administration (1-min ischemia) was followed by 30 min of reperfusion. Immunoglobulin G liposomes (IgG-L) or CSIL was also infused at 5, 10, and 20 min of ischemia, reperfused, and then prepared for histochemical staining and electron microscopy. |
| RESULTS | Recovery of left ventricular developed pressure (LVDP) of ischemic hearts treated with CSIL at 1 min of ischemia, assessed at 5 min of reperfusion ($98 \pm 14\%$), was similar to that of sham-operated hearts (100%) but was significantly greater than that of placebo-treated hearts ($12 \pm 7\%$, $p = 0.01$). The LVDP of hearts treated with CSIL at 5, 10, and 20 min was significantly greater than that with IgG-L at corresponding times ($p < 0.03$). Histochemical integrity and ultra-structural myocardial integrity were consistent with the functional data. |
| CONCLUSIONS | Preservation of myocardial viability ex vivo was achieved with CSIL therapy. The extent of preservation is proportional to the time of initiation of therapy. Beneficial effects were observed even when CSIL therapy was initiated at 20 min of global ischemia. Therefore, delayed CSIL intervention after the onset of ischemia may augment preservation of myocardial viability during reperfusion therapy. (J Am Coll Cardiol 2004;43:1683–9) |

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The major therapeutic goals of treating over one million patients with acute myocardial infarction (AMI) annually (1) are to decrease morbidity and to decrease mortality. Diagnosis and treatment should optimally occur within 4 to 6 h of chest pain (2). Irreversible myocardial injury from prolonged ischemia results in myocyte membrane lesions, the hallmark of oncotic cell death (3). Thrombolytic therapy or percutaneous transluminal coronary angioplasty can restore perfusion but does not repair the membrane lesions of the affected myocytes and is associated with concurrent reperfusion injury (4,5). To reduce oncotic cell death associated with cell membrane lesions, we developed a method to seal myocyte membrane lesions with (antimyosin) cytoskeleton-specific immunoliposomes (CSIL) (6). Anti-myosin antibody is highly specific for targeting myosin, exposed through membrane lesions in the diagnosis of AMI (7,8), myocarditis (9), and heart transplant rejection (10).

Liposomes are bilayer lipid vesicles used in diagnosis, water-based ointments, gels in cosmetics, and drug and gene delivery systems (11). Neutral liposomes are non-toxic in serum (11). They can be made target-specific with

antibodies (6). Thus, liposomes have been useful in biologic applications for several decades (11–15).

We hypothesized that lesions that developed in the ischemic myocardium would allow antimyosin on CSIL to anchor to the exposed myosin, thus sealing the lesions, followed by fusion of the liposomal lipid bilayer with the cell membrane lipid bilayer (6,16). This should result in restoration of cell membrane integrity and, if combined with reperfusion, should lead to preservation of myocardial integrity. The present study demonstrates that myocardial preservation can be achieved in a time-dependent manner with CSIL therapy in an ex vivo model of globally ischemic Langendorff perfused rat hearts.

METHODS

Myosin-specific monoclonal 2G42D7, an immunoglobulin G (IgG)-1 murine antibody with an apparent affinity of $\approx 1 \times 10^9$ l/mol (17,18), was produced in bioreactors (Cell-max QUAD, Cellco Inc., Laguna Hills, California), purified by protein A-affinity chromatography (19), and purity-assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (X-Cell Surelock Mini-Cell with NuPage gel, 10% Bis-Tris, Invitrogen, Carlsbad, California). The antibody activity was assessed by enzyme-linked immunosorbent assay (6,20).

Modification of antibody. Antimyosin antibody was modified with N-glutaryl phosphatidyl ethanolamine (NGPE; Avanti Polar Lipids, Hercules, California) (21). Briefly, 2

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Abbreviations and Acronyms

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| AMI | = acute myocardial infarction |
| CPP | = coronary perfusion pressure |
| CSIL | = cytoskeletal-specific immunoliposomes |
| IgG-L | = immunoglobulin G liposomes |
| LV | = left ventricle/ventricular |
| LVDP | = left ventricular developed pressure |
| NBT | = nitro blue tetrazolium |
| NGPE | = N-glutaryl phosphatidyl ethanolamine |
| PBS | = phosphate-buffered saline |

mg 2G4-2D7 in 2 ml of 137mmol/l NaCl, 2.7 mmol/l KCl, 8.5 Na₂HPO₄, 1.4 mmol/l KH₂PO₄, at pH 7.4 (phosphate-buffered saline [PBS]), was mixed with solid HEPES (24 mg). Then, NGPE (0.3 mg) in 0.5 ml of 0.016 mol/l octyl-glucoside in 50 mmol/l of 2-N-morpholino ethane sulfonic acid (pH 4.5) was activated with 12 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and 15 mg N-hydroxysulfosuccinimide and slowly added to the antibody solution; pH was adjusted to \approx 8.0 with 1 mol/l KOH and incubated overnight at 4°C with gentle stirring. MOPC-21 murine IgG1 was similarly modified.

Immunoliposome preparation. Unilamellar liposomes were prepared by the detergent dialysis method (22). Phosphatidyl choline (30 mg) in chloroform and cholesterol (18 mg) (1:1 molar ratio) were dried for 2 h in a rotary evaporator. Two milliliters of 50 mg octyl-glucoside/ml PBS were added to the dry lipid film, stirred, and sonicated for 5 min. Next, NGPE/antimyosin or NGPE/MOPC-21 IgG was added, stirred, and sonicated (2 min), then dialyzed against 4 l PBS (pH 7.4) overnight at 4°C. Resulting liposomes were extruded serially through 0.8-, 0.45- (Millex, Bedford, Massachusetts), and 0.2- μ m polycarbonate membrane syringe filters (6,16). The mean (\pm SD) diameter of the immunoliposomes was 200 ± 35 nm (Coulter N4+ MD Submicron Particle Size Analyzer, Coulter Electronics, Miami, Florida).

Langendorff perfused isolated hearts. The Langendorff isolated perfused heart model was used (23). The hearts of CD-1 male rats (250 to 300 g; n = 4 each group, 34 total) were excised and perfused within 25 s with non-recirculating oxygenated Krebs-Henseleit bicarbonate buffer (120 mmol/l NaCl, 25 mmol/l NaHCO₃, 1.2 mmol/l MgSO₄ 7H₂O, 5 mmol/l KCl, 1.7 mmol/l CaCl₂ 2H₂O, 10 mmol/l glucose, pH 7.4, 37°C) at a constant coronary perfusion pressure (CPP) of 80 mm Hg. Each heart immersed in 0.9% NaCl at 37°C in a water-jacketed chamber was paced at 300 beats/min (5 Hz). The left ventricular (LV) end-diastolic pressure was set at 10 mm Hg, utilizing a water-filled balloon-tipped catheter attached to a pressure transducer. Baseline hemodynamic measurements were recorded on a strip-chart recorder (Hewlett-Packard 7754A) for a 10-min stabilization period. Global ischemia (25 min) was initiated by decreasing the CPP to zero within 60 s. A 2-ml aliquot of freshly prepared 1 mg NGPE/antimyosin/CSIL, 1 mg non-specific IgG lipo-

somes (IgG-L), or placebo (PBS) was infused at various times during ischemia. A 3-ml syringe was used to deliver various reagents via a three-way stopcock placed 8 cm above the aorta, enabling injection without turning on the perfusion pump, while maintaining the ischemic condition. During ischemia, the heart was paced for 5 min at zero CPP to ensure adequate myocardial injury, then left unpaced for 20 min longer. Perfusion was restored to 80 mm Hg, and pacing was restarted at 3 min of reperfusion. Hemodynamic measurements were recorded for 30 min of reperfusion. Hearts were weighed, sectioned transversely (5 or 6 slices), and stained for 20 min in 0.05% nitro blue tetrazolium (NBT; Fisher Scientific, Fair Lawn, New Jersey) at 60°C (12). Sham-instrumented hearts underwent an identical procedure without ischemia.

This protocol was approved by the Institutional Animal Care and Use Committee, Northeastern University (Boston, Massachusetts) and conforms to the guidelines specified in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Assessment of myocardial preservation. Myocardial function was assessed as the left ventricular developed pressure (LVDP)—the difference between the LV end-systolic and diastolic pressures, measured at pre-ischemic, ischemic, and post-ischemic times (5, 10, 15, 20, 25, and 30 min of reperfusion). The LVDP recovery during reperfusion was calculated as the mean percent LVDP of the pre-ischemic baseline values.

A single-blinded histochemical analysis was determined by computer planimetry (Adobe Photoshop version 4.0) of the digital photographs of NBT-stained heart slices. The digital images were assigned numbers; the total and infarct areas of the right ventricular and LV slices were quantitated by computer planimetry after adjusting the brightness and contrast for optimal differentiation of the infarcted regions (Fig. 1A). The single-blinded code was broken, and infarct sizes were correlated to various treatments.

For ultra-structural assessment, a sample from the mid-portion of the LV of each heart was cut into smaller pieces (\sim 0.5 mm³); prefixed in 3% glutaraldehyde, 0.1 mol/l sodium cacodylate, and 2% formaldehyde; postfixed in 1% osmium tetroxide and 0.1 mol/l sodium cacodylate; dehydrated in a graded series of ethanol; and embedded in Spurr's resin and polymerized overnight at 60°C. Ultra-thin sections (gold-silver) were cut using a diamond knife (DuPont Company) and ultramicrotome (Reichert Ultracut E, Austria), and the sections were contrasted with uranyl acetate and lead citrate (24) and examined with a JEOL (JEM1010) transmission electron microscope.

Two micrographs each (\times 6,000 magnification) from two hearts of each group were randomly obtained, and all mitochondria in the micrographs were quantitated by computer planimetry. From each group, 225 ± 12 (mean \pm SEM) mitochondria were analyzed.

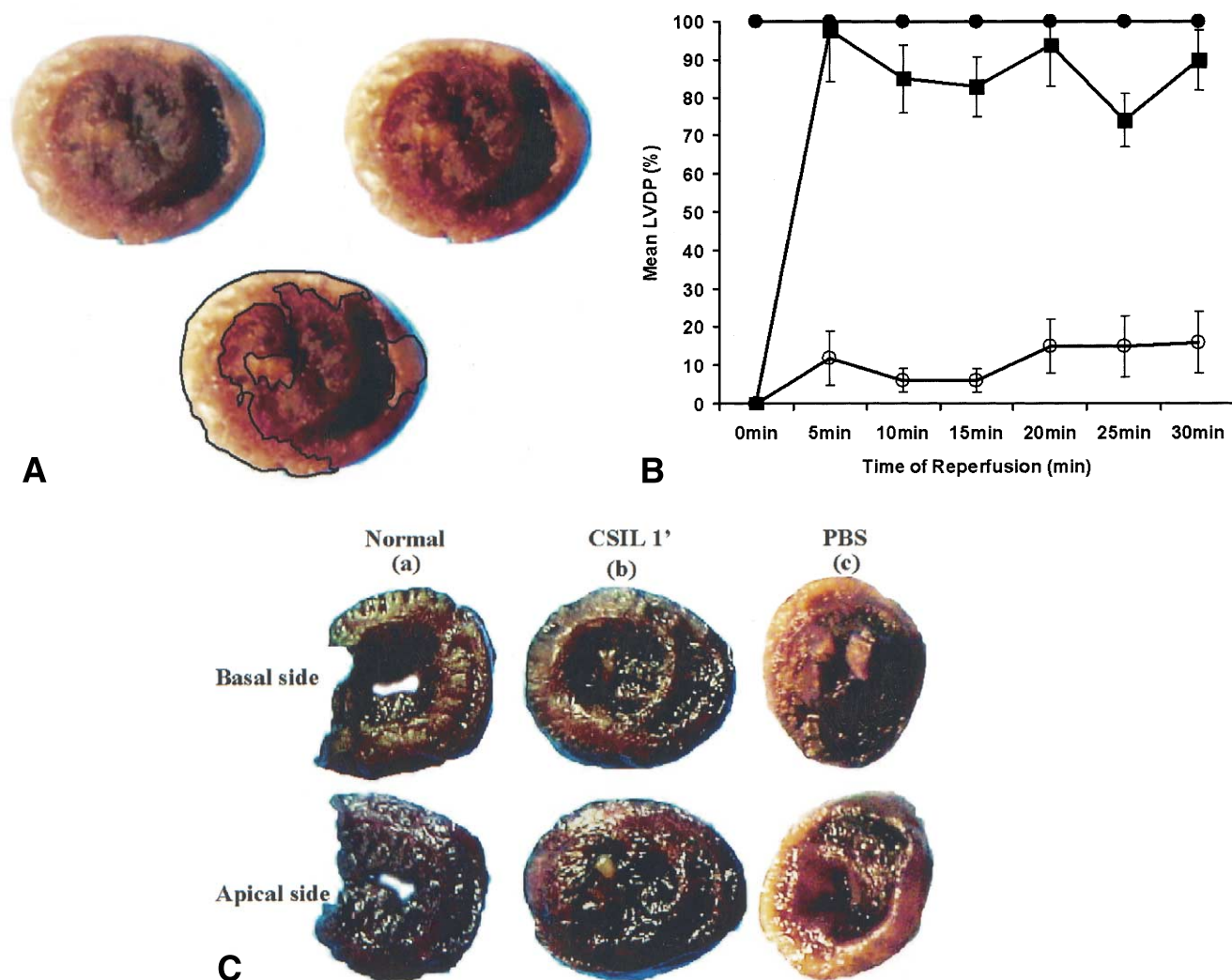


Figure 1. (A) A digital photograph of a representative nitro blue tetrazolium-stained heart slice (top left), a color-contrasted version to highlight the infarcted regions (top right), and an outline of the planimetered infarct area for infarct sizing (bottom). (B) Mean left ventricular developed pressures (LVDPs) (% pre-ischemic values) at 5 to 30 min of reperfusion after 25 min of global ischemia of hearts treated with cytoskeletal specific immunoliposomes (CSIL) at 1 min of ischemia (CSIL 1') (squares), PBS (open circles), or sham-instrumented hearts (solid circles). (C) Representative digital photographs of nitro blue tetrazolium-stained mid-slices of normal hearts (a) or hearts treated with CSIL 1' (b) or PBS (c).

Statistical analyses. Results are reported as the mean value \pm SEM. Wilcoxon rank-sum distribution (25) was used to determine significant differences of the mean values for all statistical comparisons in this report. Alpha was set at ≤ 0.05 .

The LVDP of treatment groups (at 5 min of reperfusion, or the overall mean value of individual mean LVDPs at 5, 10, 15, 20, 25, and 30 min of each group for total time-function curves) was compared. The same statistical analysis was employed in the time response study, the comparison of single-blinded histochemical infarct sizes, the mean mitochondrial sizes, and LVDP of hearts at the plateau phase (between 20 and 30 min of reperfusion).

RESULTS

Treatment at 1 min of ischemia. Treatment with CSIL initiated at 1 min of global ischemia resulted in functional

recovery in isolated rat hearts by 5 min of reperfusion. This LVDP ($98 \pm 14\%$) was similar to that of sham controls, but was greater than that of hearts treated with placebo ($12 \pm 7\%$, $p = 0.01$) (Fig. 1B).

The total time-function LVDP curves showed that recovery after CSIL treatment at 1 min of ischemia ($87 \pm 6\%$) was not significantly different from that of the sham group, but was greater than that of placebo-treated hearts ($12 \pm 2\%$, $p = 0.01$).

The sufficiency of 25 min of global ischemia to induce extensive myocardial injury is demonstrated by the extensive lack of NBT staining in heart slices of placebo controls (Fig. 1C, panel c). Hearts treated with CSIL at 1 min of ischemia were almost normally stained on both the basal and apical sides of the slices (Fig. 1C, panel b) and were similar to the untreated normal heart slices (Fig. 1C, panel a).

Time response. The mean LVDP of the total time-function curve of CSIL-treated hearts at 5, 10, and 20 min

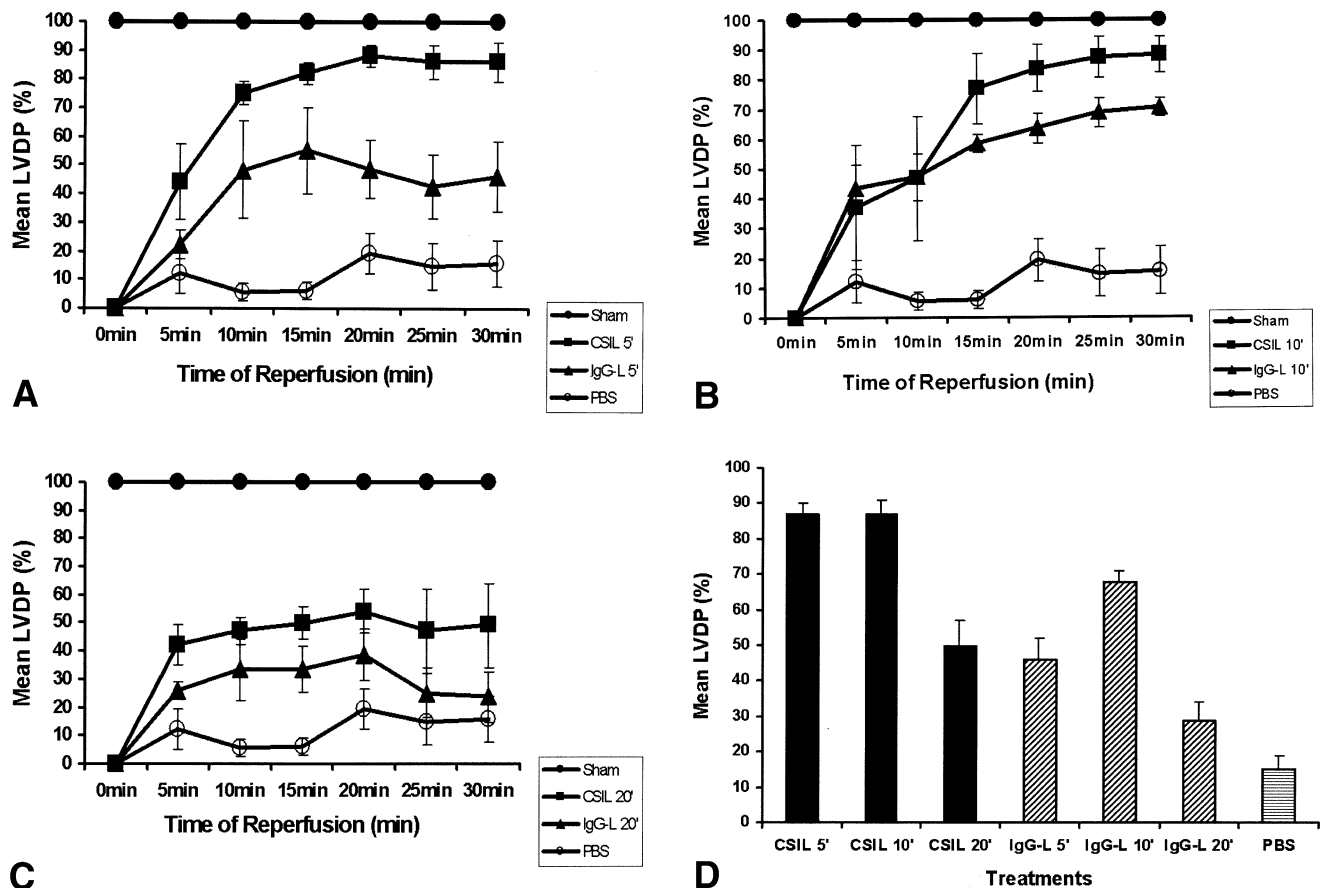


Figure 2. (A) Mean left ventricular developed pressure (LVDP) during reperfusion of hearts treated with cytoskeletal specific immunoliposomes (CSIL) at 5 min of ischemia (CSIL 5'), immunoglobulin G liposomes (IgG-L) at 5 min of ischemia (IgG-L 5'), phosphate buffered saline (PBS), or sham instrumentation. (B) Mean LVDP during reperfusion of hearts treated with CSIL or IgG-L at 10 min of ischemia (CSIL 10' and IgG-L 10'), PBS, or sham. (C) Mean LVDP during reperfusion of hearts treated with CSIL or IgG-L at 20 min of ischemia (CSIL 20' and IgG-L 20'), PBS, or sham. (A through C) Sham = solid circles; CSIL = squares; IgG-L = triangles; PBS = open circles. (D) Mean LVDP assessed at 20 to 30 min of reperfusion (plateau phase) in hearts treated with CSIL, IgG-L, or PBS.

of ischemia ($77 \pm 3\%$, $70 \pm 12\%$, and $48 \pm 8\%$, respectively) was less than that of the sham-operated hearts but greater than that of IgG-L ($44 \pm 7\%$, $58 \pm 4\%$, and $30 \pm 4\%$, respectively) or placebo-treated hearts ($12 \pm 2\%$, $p = 0.01$) (Figs. 2A to 2C).

A sequential delay of 5, 10, and 15 min in the recovery of function to near normal LVDP was observed in hearts treated with CSIL at 1, 5, and 10 min of ischemia, respectively ($p = \text{NS}$), but all LVPD values were greater than those of hearts treated with CSIL at 20 min ($p \leq 0.05$). The time-function curves for hearts treated with CSIL at 1 and 5 min of ischemia were greater than those at 20 min ($p = 0.01$).

Functional assessment at 20 to 30 min of reperfusion. In all treated hearts, mean LVDP recovery reached a plateau by 20 min of reperfusion (Figs. 1B, 2A to 2C). The mean plateau LVDP in hearts treated with CSIL at 5 and 10 min of ischemia ($87 \pm 3\%$ and $87 \pm 4\%$, respectively) was greater than that of the corresponding IgG-L controls ($46 \pm 6\%$ and $68 \pm 3\%$, $p = 0.01$ and 0.02 , respectively). The

plateau LVDP of hearts treated with CSIL at 20 min of ischemia ($50 \pm 7\%$) was greater than that of IgG-L controls ($29 \pm 5\%$), but was not statistically different ($p = 0.1$); however, it was significantly greater than that of placebo controls ($15 \pm 4\%$, $p \leq 0.01$) (Fig. 2D). No difference in LVDP was observed between hearts treated with CSIL at 5 and 10 min of ischemia.

Single-blinded histochemical assessment. Minimal unstained areas of the myocardium, indicative of minimal injury, were observed in hearts treated with CSIL at 5 min of ischemia (Fig. 3A, panel a). Myocardial injury increased with longer delays of CSIL treatment (at 10 and 20 min) (Fig. 3A, panels b and c). Hearts treated with IgG-L at 5, 10, and 20 min of ischemia (Fig. 3A, panels d to f) showed more extensive injury than did corresponding CSIL-treated hearts. Infarct sizes of hearts treated with IgG-L at 5, 10, or 20 min of ischemia ($39 \pm 4\%$, $35 \pm 7\%$, and $45 \pm 6\%$, respectively) were the same (Fig. 3B). Infarct sizes of hearts treated with CSIL at 1, 5, and 10 min ($4 \pm 1\%$, $8 \pm 3\%$, and $6 \pm 2\%$, respectively) were similar to sham hearts ($3 \pm$

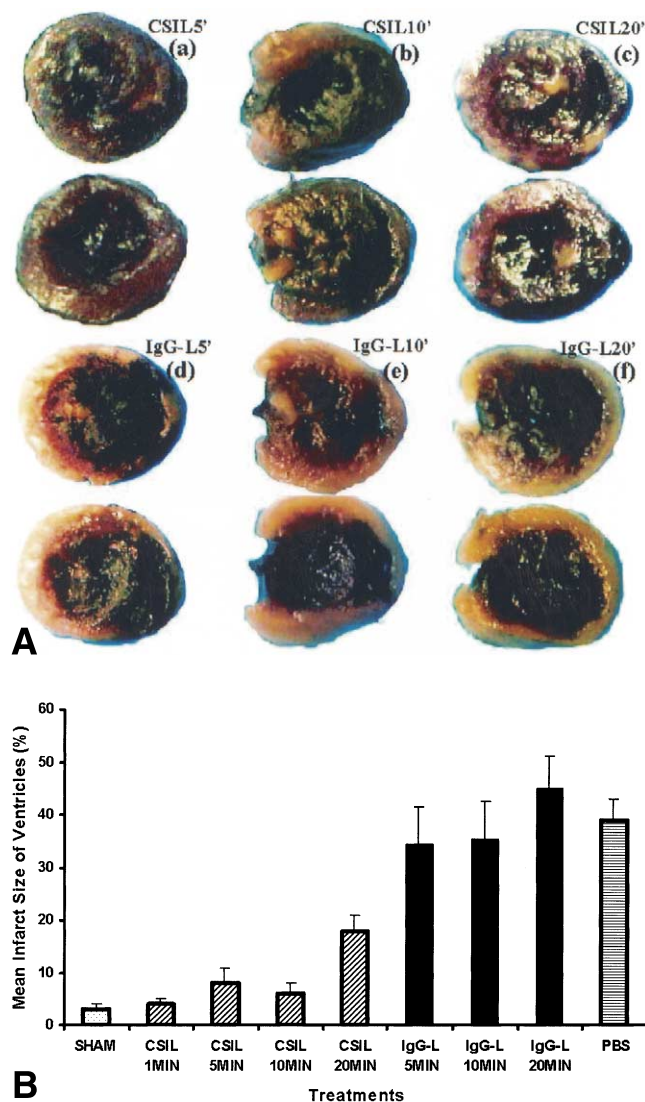


Figure 3. (A) Digital photographs of mid-slices of nitro blue tetrazolium-stained hearts treated with cytoskeletal specific immunoliposomes (CSIL) at 5 (a), 10 (b), and 20 (c) min of ischemia or immunoglobulin G liposomes (IgG-L) at 5 (d), 10 (e), and 20 (f) min of ischemia. (B) Mean infarct sizes are expressed as the percentage of right and left ventricles of hearts treated with CSIL, IgG-L, phosphate buffered saline (PBS), or sham instrumentation.

2%, $p = \text{NS}$), but were smaller than hearts treated with CSIL at 20 min ($19 \pm 3\%$, $p \leq 0.05$). Infarct sizes of hearts treated with CSIL at 5, 10, and 20 min of ischemia were smaller than those treated with IgG-L at the corresponding times (Fig. 3B).

Mitochondrial size. The average size of normal mitochondria ($1,441 \pm 146$ [mean number of pixels \pm SEM]) was similar to that of hearts treated with CSIL at 1, 5, 10, and 20 min of ischemia ($1,496 \pm 103$, $1,496 \pm 66$, $1,845 \pm 147$, and $1,504 \pm 101$, respectively; $p = \text{NS}$) (Fig. 4). The mean mitochondria size of hearts treated with IgG-L at 5, 10, and 20 min of ischemia ($2,294 \pm 95$, $2,387 \pm 119$, and $2,667 \pm 37$, respectively) or placebo ($2,234 \pm 270$) was greater than that of CSIL-treated hearts ($p \leq 0.05$).

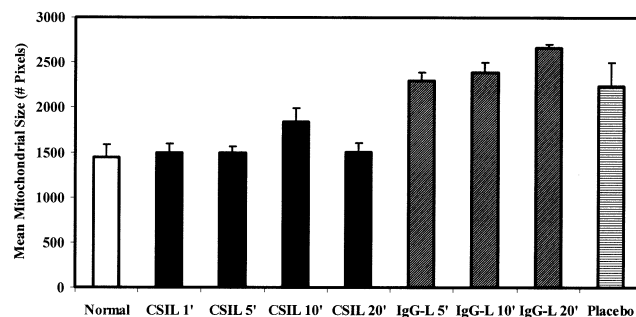


Figure 4. Mean pixel sizes of mitochondria in normal hearts, those treated with cytoskeletal specific immunoliposomes (CSIL) and immunoglobulin G liposomes (IgG-L) at various times after initiation of ischemia, and placebo-treated hearts.

DISCUSSION

The current study reports on the preservation of myocardial viability in ex vivo adult ischemic hearts treated with CSIL. Treatment with CSIL initiated at 1 min of ischemia resulted in apparent complete preservation of function, viability, and mitochondrial integrity. A time-dependent relationship between CSIL administration and myocardial preservation was evident. This beneficial effect is maintained even when treatment was initiated at longer durations of global ischemia. Thus, CSIL treatment need not be initiated immediately but may be rendered subsequent to the onset of AMI.

Study design. In the coronary artery-occluded Langendorff rat heart model, irreversible injury occurred within 20 min of ischemia (5). Disruption of the sarcolemma was observed in canine hearts subjected to 15 (26,27) to 20 min of coronary occlusion and reperfusion (28). The model of 25 min of global ischemia and 30 min of reperfusion was chosen to provide more extensive irreversible myocardial injury than the 20-min ischemia model. The extent of myocardial injury is augmented by pacing the hearts for an additional 5 min during global ischemia, which resulted in $\sim 30\%$ infarction of the total ventricles.

Assessment of myocardial preservation. The extent of recovery indicated by hemodynamic, histochemical, and mitochondrial ultra-structure indicates that myocardial preservation after CSIL treatment was achieved.

Functional recovery. Preservation of myocardial viability with early CSIL treatment is consistent with clinical observations that maximal therapeutic benefits are associated with early intervention (29). This benefit persisted even with late administration of CSIL. However, there is a time-dependent delay in the recovery to near normal LVDP with a delay in the initiation of CSIL therapy (Figs. 1B, 2A, and 2B), which may be due to the need for more extensive myocardial cell membrane lesion sealing. In isolated perfused hearts, ventricular dysfunction may be due to myocardial stunning or lethal cell injury (30). Recovery from stunned to normal myocardium requires 24 to 48 h in the in vivo reperfused heart with coronary artery occlusion of 2 to 20 min (31). Stunning is related to decreased availability of

adenosine triphosphate (ATP) to the myofibrils; injury of the contractile apparatus; alterations in calcium homeostasis with calcium overload; and burst of free radicals (30). The earliest consequence of severe ischemia is contractile dysfunction, occurring within 6 to 10 s of ischemia (30). In the Langendorff perfused ischemic rat hearts, ATP concentrations decrease rapidly to 60% in the first minute, with a rapid secondary decrease by 13 min due to contracture (32). Treatment with CSIL resulted in a rapid recovery of function; whether this is due to the reversal of myocardial stunning must await further studies.

Greater functional recovery observed in IgG-L-treated hearts than in placebo hearts is not consistent with the infarct size data. The presence of IgG-L in the ischemic myocardium may provide a non-specific mechanism of increasing myocardial function. Dobrinina et al. (33) showed that neutral liposomes preserved liver integrity in rats subjected to hepatotropic poisons by non-specific mechanisms. However, histochemical infarct size data do not support this hypothesis (Fig. 3B). The liposomes of IgG may temporarily plug membrane lesions without fusion with the cell membrane, ultimately leading to myocardial cell death, as determined by NBT. Nonetheless, the LVDP of IgG-L-treated hearts was still lower than that of CSIL-treated hearts.

Myocardial viability. Loss of tetrazolium staining after ischemic injury occurs due to loss of cofactor NADH and enzymatic activity. The total NAD and NADH contents are relatively stable in the early phases of ischemia but decrease after irreversible myocardial injury (30). Hearts treated with CSIL at 1 min of ischemia were similar to sham hearts that have minimal injury, a result of the insertion of the thebesian drainage port into the apex. Hearts treated with CSIL at earlier times (1, 5, and 10 min) showed extensive NBT staining, consistent with maximal myocardial preservation. Hearts treated with CSIL at 20 min resulted in increased regions of negative NBT staining, indicating that myocardial recovery was not complete (Fig. 3A, panel c). However, the extent of histochemical myocardial injury was consistent with the extent of recovery of function in these hearts.

Similar to the recovery of LVDP of hearts treated with CSIL, NBT staining also indicated that there was greater myocardial salvage in all hearts treated with CSIL than in IgG-L-treated controls (Fig. 3B).

Ultra-structural morphologic integrity. Cessation of mitochondrial electron transport has been observed 2 s after the onset of global ischemia in isolated rat hearts (34). Pronounced mitochondria swelling with loss of cristae, development of amorphous matrix densities, and breaks in the sarcolemma are seen in irreversibly injured myocytes (30). Increased mitochondrial size is an early indication of ischemia. In hearts with 3 to 15 min of ischemia, morphologic injury is reversible, yet some mitochondria remained swollen even at 20 min of reperfusion (30). Therefore, a majority of the mitochondria in the ischemic myocardium is

expected to be edematous at 30 min of reperfusion. However, early CSIL treatment of ischemic hearts (1 and 5 min) resulted in normal mitochondrial size, smaller than that of CSIL-treated hearts at later times. The results of mitochondrial size determination are compatible with the delay in the recovery of function with later administration of CSIL. Thus, mitochondrial ultra-structural data agree with the functional and histochemical data.

Preservation of cell membrane integrity after CSIL intervention resulted in a faster recovery of function and a reduction in infarct size. Whether this is associated with prevention of the influx of extracellular Ca^{2+} that is associated with ischemia and reperfusion was not assessed in this study. However, uncontrolled influx of Ca^{2+} into the cytosol occurs after reperfusion and results in rigor (35,36). Treatment with CSIL enabled globally ischemic hearts to return to near normal function within 15 min of reperfusion, which is consistent with the prevention of the occurrence of uncontrolled myocardial Ca^{2+} overload. The absence of mitochondrial swelling and the return of function to near normal in CSIL-treated hearts are also consistent with the maintenance of Ca^{2+} homeostasis.

Cell membrane lesion sealing with neutral immunoliposomes may also reduce injury mediated by acid and oxidative stress. However, plain liposomes in serum-free perfusate may augment this injury. Plain liposomes may mimic fatty acids that inhibit enzymes, which may result in the uncoupling of oxidative phosphorylation. They may also act as detergents, resulting in additional disruption of cell membranes (37).

Study limitations. Our model of Langendorff perfused hearts used protein-free oxygenated perfusion buffer. It is a non-working heart model; therefore, the ischemic myocytes are not subjected to additional stresses. Furthermore, prolonged periods of reperfusion >30 min cannot be investigated in this model, because of the inability to maintain long-term steady-state myocardial function *ex vivo*. Future *in vivo* studies should permit investigation of prolonged ischemia and reperfusion with CSIL therapy.

The results presented may not necessarily imply that CSIL therapy may be beneficial in a clinical scenario of AMI. Furthermore, no conclusions can be drawn regarding the impact of CSIL therapy on apoptotic myocardial cell death.

Proposed mechanism of cell membrane lesion sealing. Although no direct *ex vivo* or *in vivo* evidence of cell membrane lesion sealing was demonstrated in the current study, the mechanism may be inferred from previous reports (6,16). Cardiocytes treated with fluorescent lipid-incorporated CSIL showed integration of the fluorescent lipid into the cell membranes. Furthermore, fusion of CSIL with the cell membrane may be inferred from gene transfection studies. Reporter genes entrapped in the intraliposomal cavities of CSIL successfully transfected hypoxic cardiocytes. Only hypoxic cardiocytes treated with CSIL, and not with IgG-L, plain liposomes, nor placebo, resulted

in highly efficient gene transfection (16). Furthermore, if preservation of myocardial viability were by non-specific mechanisms, IgG-L treatment should result in the same extent of myocardial salvage.

Conclusions. This study supports the hypothesis that cardiac cell membrane lesion sealing with CSIL resulted in preservation of myocardial viability, as determined by function, histochemistry, and ultra-structural morphology. There is also a time response to myocardial preservation with CSIL therapy. Early CSIL intervention after the onset of ischemia resulted in almost complete myocardial recovery. Even when the intervention was initiated at 20 min of global ischemia, myocardial preservation was still greater than that seen in hearts with IgG-L or placebo treatment. Therefore, CSIL therapy in AMI may result in beneficial therapeutic outcome.

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